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## Growth performance and biochemical composition of nineteen microalgae collected from different Moroccan reservoirs

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### Abstract

Macro- and microalgae have recently received much attention due to their valuable chemical constituents. In order to increase existing data, the authors studied nineteen microalgal species isolated from different reservoirs in the Fez region (northern Morocco), undertaking experiments to determine for each species its specific growth rate, total amounts of proteins, carbohydrates and lipids and the influence of the growth phase on these chemical constituents. The growth rates of the nineteen studied species of microalgae showed a wide interspecies variation, ranging from 0.27 d<sup>-1</sup> for *Chlamydomonas nivalis* to 3.64 d<sup>-1</sup> for *Chlorococcum wemmeri*. Protein, carbohydrate and lipid contents also varied greatly between taxa and within genera. *Ankistrodesmus falcatus*, *Chlamydomonas nivalis*, *Chlorococcum* sp., *Fragilaria ulna*, *Scenedesmus protuberans*, and *Synechocystis aquatilis* tended to synthesize proteins, the concentrations exceeding 20% of dry weight (DW). *Ankistrodesmus falcatus*, *Ankistrodesmus* sp., *Chlorococcum wemmeri*, *Coenocystis* sp., *Isocystis* sp., *Lyngbya bergei*, *Oscillatoria amphibia*, *Polytoma papillatum*, *Scenedesmus protuberans*, *Scenedesmus* sp. and *Synechocystis aquatilis* showed a high capacity for lipid storage, higher than 20% DW. For carbohydrate contents, only *Scenedesmus protuberans* and *Scenedesmus quadricauda* showed an excessive level, compared to other scanned species, with 29.21% and 24.76% DW respectively. Green algae, *Scenedesmus protuberans* for example, generally tend to synthesize proteins, with a content reaching 45% DW, while blue-green algae, for example *Lyngbya bergei*, tend to synthesize lipids, with a content reaching 50% DW.

**Keywords:** Microalgae, biochemical composition, growth rate, freshwater.

### Introduction

With over 200,000 species currently identified (Koller *et al.*, 2012), microalgae are a diverse group of organisms both biologically and ecologically (Aleya, 1989). These organisms are well known for their vast potential in a wide variety of applications and constitute promising bio-catalysts to be used in the growing field of “white biotechnology”. Microalgae can be used to produce a wide range of metabolites such as proteins, lipids, carbohydrates, pigments, cosmetics, vitamins for health, food and food additives, but also in diverse facets of “green energy” production, an area which has recently garnered much interest in different fields of research (Stengel *et al.*, 2011). In addition, these microorganisms can play an important role in bioremediation of

wastewater and carbon dioxide sequestration (Khattabi *et al.*, 2006). This wide range of applications is due to the microalgal photosynthesis potential, which makes more efficient use of sunlight energy than higher plants (Reynolds, 1997). Also, some of these microalgae can double their biomass within 24 hours (Aleya *et al.*, 2011) or can grow throughout the year, with possible continuous cultivation and harvest (Chen *et al.*, 2011). In fact, they may be good food producers for both humans and animals due to their particularly interesting biochemical characteristics: high protein, good fat and amino acids (Becker, 1988). Many systems of mass production have been developed for various species, including *Scenedesmus quadricauda* (Simmer, 1969), *Scenedesmus acutus* (Gross *et al.*, 1982), *Scenedesmus obliquus* (El-Fouly *et al.*, 1985) and *Chlorella* sp. (Hsieh & Wu, 2009). How-

ever, it should be noted that only a limited number of species is currently being exploited, though given the number identified to date, a more comprehensive screening of species with a food or industrial potential is required. A number of key criteria must be taken into consideration, such as the rapid growth of selected strains and an interesting biochemical composition (high protein levels or potential for products with high added value). In order to increase the existing data, nineteen microalgal species were studied, isolated from three reservoirs in the region of Fez (northern Morocco). The purpose of this study was twofold: (1) to determine the specific growth rate of each species along with their total amounts of proteins, carbohydrates and lipids, and (2) to examine the influence of the growth phase on these chemical constituents.

## Materials and methods

### Microalgae sampling and microalgae isolation

Samples of selected species were collected at different dates from August 2009 to November 2010 (Table 1) using a net with a 45 µm mesh size, from three reservoirs in the region of Fez (northern Morocco, Fig. 1).

The culturing of isolated microalgal species is well established, beginning with the works of Beijerinck (1890) and Miquel (1890-1892), as was noted by Andersen (2005) who also pointed out that some species are easy to isolate and cultivate (often called weeds), whereas others are diffi-

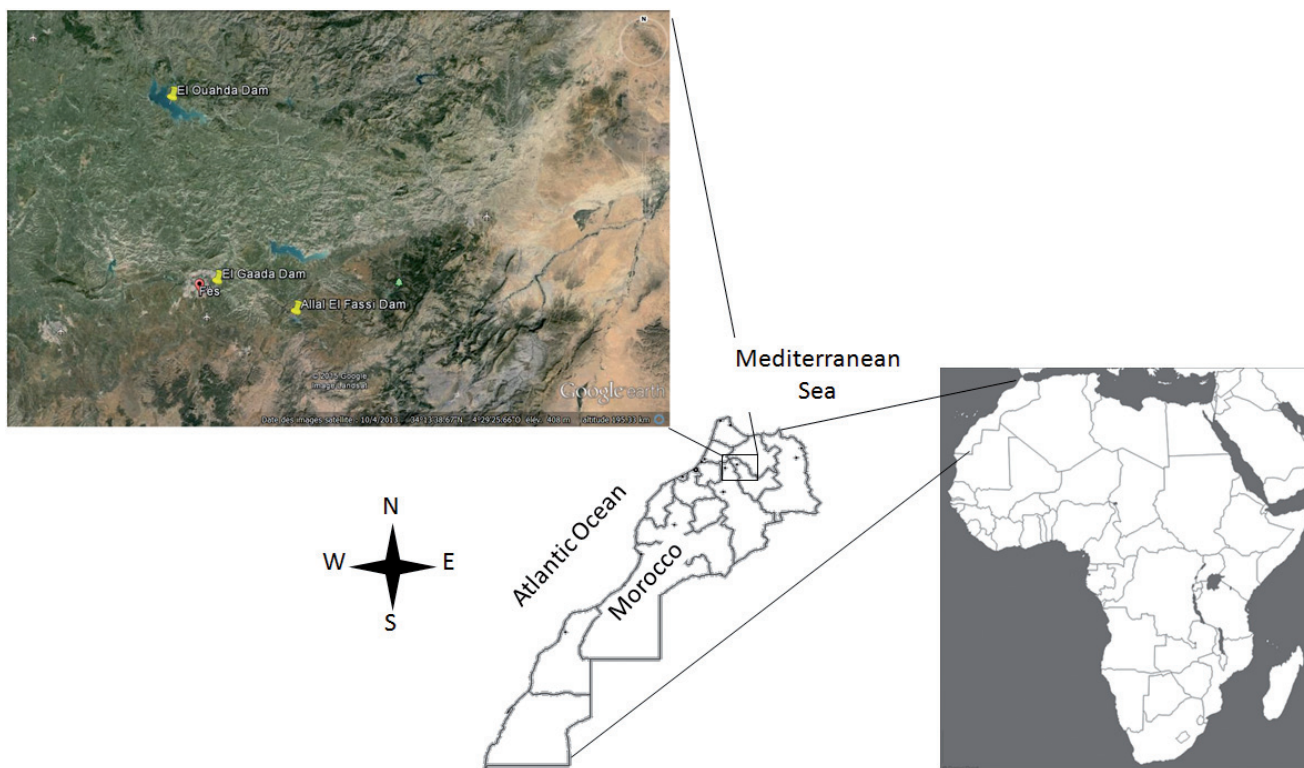
cult or seemingly impossible to grow. The first step toward a successful isolation is understanding and reproducing the naturally occurring environmental conditions (Andersen, 2005). Freshwater algae collected in non-winter months are frequently less sensitive to temperature, but their pH or alkalinity may be high. A good taxonomic knowledge of the target species may be essential: Diatoms require silica, Euglenoids often require ammonia and some genera (e.g., the Prymnesiophyceae *Chrysochromulina*) require selenium. Mixotrophic species (e.g., certain dinoflagellates and chrysophytes) often require a bacterial food source, and colorless phagotrophic species (e.g., *Pfiesteria*) may require a eukaryotic food source. Thus, to isolate algal strains after harvesting phytoplankton, a first inoculum was grown in test tubes containing liquid culture medium "Synura" proposed by Brunel-Delclaux and Guerrin (1980) under light intensity equal to 300 µmol photons m<sup>-2</sup> s<sup>-1</sup>, with a temperature regime of 25/20°C (day/night) and using a 16/8 (light/dark) photoperiod cycle. The second step toward a successful isolation involves the elimination of contaminants, especially those that can outcompete the target species. Single-cell isolation by micropipette or agar streaking was used. The experimental design is summarized in Figure 2.

### Microalgae cultivation

For biochemical analyses algae were grown in Erlenmeyer flasks containing three liters of sterile culture

**Table 1.** Microalgae species list, collection dates and sampling sites' location.

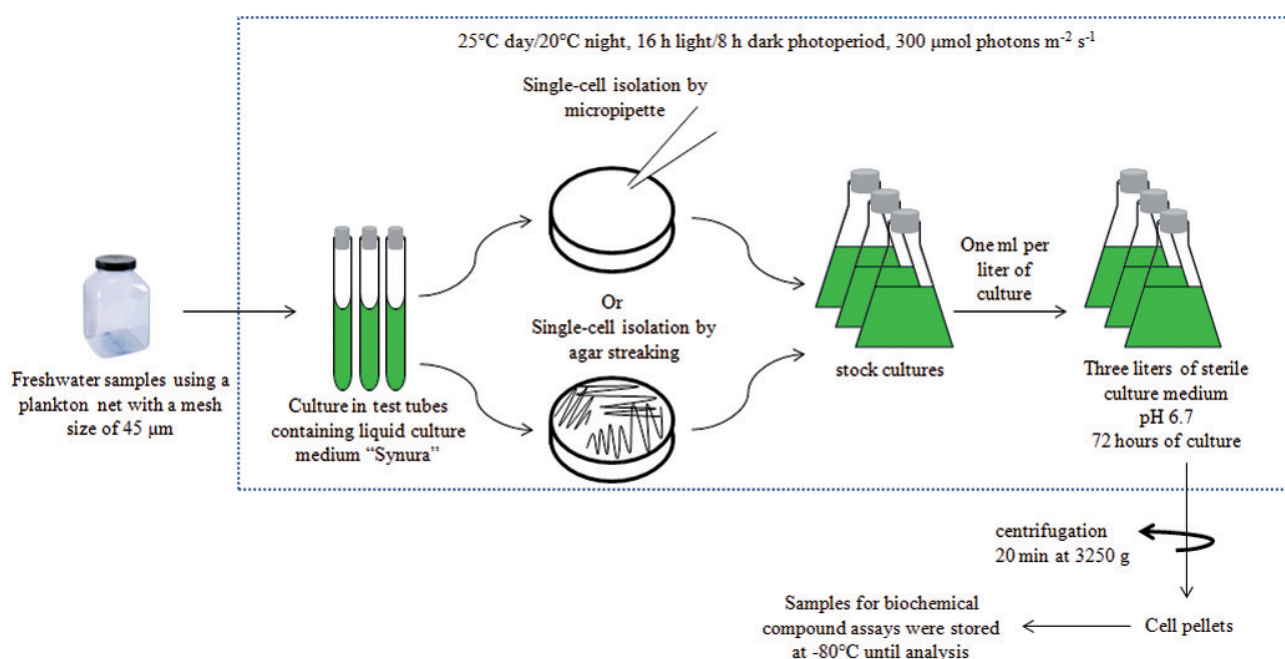
Microalgal species	Sampling dates	Sampling sites
<i>Lyngbya bergei</i>	8/5/2009	
<i>Isocystis</i> sp.	8/5/2009	
<i>Polytoma papillatum</i>	8/5/2009	
<i>Hyaloraphidium contortum</i>	12/6/2009	
<i>Ankistrodesmus falcatus</i>	12/6/2009	
<i>Ankistrodesmus</i> sp.	12/6/2009	
<i>Chlorococcum</i> sp.	5/4/2010	El Gaada dam
<i>Chlorococcum wemmeri</i>	5/4/2010	34°01'02.40" N
<i>Selenastrum bibraianum</i>	5/9/2010	4°57'12.48" O
<i>Scenedesmus protuberans</i>	6/5/2010	
<i>Scenedesmus falcatus</i>	6/5/2010	
<i>Synechocystis aquatilis</i>	8/5/2010	
<i>Scenedesmus quadricauda</i>	8/5/2010	
<i>Scenedesmus</i> sp.	8/11/2010	
<i>Coenocystis</i> sp.	10/30/2009	Allal El Fassi dam
<i>Oscillatoria amphibia</i>	10/30/2009	33°54'41.67" N
<i>Fragilaria</i> sp.	1/31/2010	4°38'27.51" O
<i>Chlamydomonas ovalis</i>	8/11/2010	El Ouahda dam
<i>Chlorella</i> sp.	8/11/2010	34°34'34.79" N
		5°07'43.50" O



**Fig. 1:** Geographic location of the three dams in the Fez region (northern Morocco) where the algal sampling was conducted. Map source: Google Earth.

medium, containing  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  36.97 mg.  $\text{l}^{-1}$ ;  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  0.76 mg.  $\text{l}^{-1}$ ;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  36.76 mg.  $\text{l}^{-1}$ ;  $\text{Na}_2\text{EDTA}$  2.18 mg.  $\text{l}^{-1}$ ;  $\text{NaHCO}_3$  12.6 mg.  $\text{l}^{-1}$ ;  $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$  28.42 mg.  $\text{l}^{-1}$ ;  $\text{K}_2\text{HPO}_4$  4.3 mg.  $\text{l}^{-1}$ ;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.011 mg.  $\text{l}^{-1}$ ;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  0.090 mg.  $\text{l}^{-1}$ ;  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  0.005 mg.  $\text{l}^{-1}$ ;  $\text{BO}_2\text{H}_3$  0.500 mg.  $\text{l}^{-1}$ ;  $\text{Na}_2\text{MoO}_4$  0.003 mg.  $\text{l}^{-1}$ . The culture medium composition was adjusted to pH 6.7 and then ster-

ilized by autoclave. The media were inoculated in three replicates with exponentially growing algae from stock cultures at 1 ml per liter of culture. The samples needed for the different analyses were collected every 72 hours at the same time of the day, to minimize the effects of circadian variations of certain compound contents. The algal cultures were harvested for biochemical compound assays



**Fig. 2:** Schematic representation of experimental design.



by centrifugation for 20 min at 3250 g (Aleya, 1992). The microalgae pellets were cleaned twice with 0.17 M NaCl to remove all growth medium from cells (Bligh & Dyer, 1959) and then stored at -80°C until analysis.

### Identification, biovolume and growth rate of sampled microalgae

Algal taxa were identified with various keys, while biovolumes were calculated from cell dimensions (Hillebrand *et al.*, 1999).

The growth rate of each species was determined using the following equation (1):

$$\mu = \ln(N/N_0) / (t - t_0) \quad (1)$$

Where  $N_0$  is the cultivated biomass at time  $t_0$  (beginning of the exponential phase);  $N$  is the harvested biomass at time  $t$  (ending of the exponential phase) and  $t - t_0$  is the exposure time between  $N_0$  and  $N$  (days).

### Biochemical composition

Total protein was calculated from the total Kjeldahl nitrogen ( $\times 6.25$ ) used to convert it to protein (Renaud *et al.*, 1999). Total carbohydrates were determined by spectrophotometry, using the phenol-sulphuric method and glucose as the standard, as indicated by Dubois *et al.* (1956). The lipid fraction was extracted from the biomass with the Bligh & Dyer (1959) method, obtaining a miscible system consisting of the sample water content and a mixture of chloroform and water. Total lipid concentration was determined gravimetrically from the chloroform extract, evaporating the chloroform in a nitrogen atmosphere and subsequently drying it to constant weight in a vacuum oven.

### Statistical analysis

Two-way ANOVA and the Bonferroni test for assessing differences in mean values were performed using Prism 5 (GraphPad Software). Significance level was set at  $p < 0.05$ .

## Results

### Growth rate

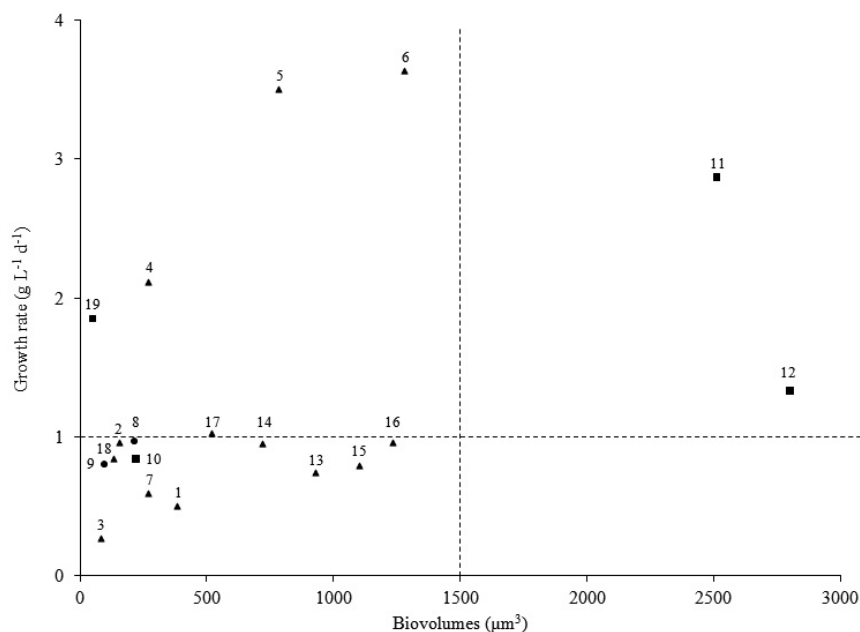
While the growth rates varied from 0.27 d<sup>-1</sup> for *Chlamydomonas nivalis* to 3.64 d<sup>-1</sup> for *Chlorococcum wemmeri*, individual biovolumes fluctuated from 50  $\mu\text{m}^3$  for *Synechocystis aquatilis* to 2800  $\mu\text{m}^3$  for *Oscillatoria amphibian* (Table 2). Figure 3 shows the dispersion of the studied microalgal species according to growth rate and individual biovolume. Arbitrarily, the species having a biovolume less than 1500  $\mu\text{m}^3$  can be seen as 2 groups; the first includes the majority of the studied strains (13) with a growth rate less than 1 d<sup>-1</sup>, while the second group (4 strains) has a growth rate higher than 1 d<sup>-1</sup>. A third group, with a growth rate and individual biovolume higher than 1 d<sup>-1</sup> and 1500  $\mu\text{m}^3$ , respectively, includes only the blue-green algae *Lyngbya bergei* and *Oscillatoria amphibia*.

### Variation in the biochemical composition according to species growth phases

Protein, carbohydrate and lipid contents measured in the different microalgae are shown in Figures 4, 5 and 6, respectively. Values are within the ranges defined in Table 3, regardless of the growth phase. Species with

**Table 2.** Biovolumes and growth rates of the studied microalgal species

	Microalgal species	Algae groups	Biovolumes ( $\mu\text{m}^3$ )	Growth rate (d <sup>-1</sup> )
1	<i>Ankistrodesmus falcatus</i>	Green	384	0.5
2	<i>Ankistrodesmus</i> sp.	Green	155	0.96
3	<i>Chlamydomonas nivalis</i>	Green	82	0.27
4	<i>Chlorella</i> sp.	Green	268	2.12
5	<i>Chlorococcum</i> sp.	Green	783	3.51
6	<i>Chlorococcum wemmeri</i>	Green	1,280	3.64
7	<i>Coenocystis</i> sp.	Green	270	0.59
8	<i>Fragilaria</i> sp.	Diatom	214	0.97
9	<i>Fragilaria ulna</i>	Diatom	95	0.8
10	<i>Isocystis</i> sp.	Blue-green	220	0.84
11	<i>Lyngbya bergei</i>	Blue-green	2,512	2.87
12	<i>Oscillatoria amphibia</i>	Blue-green	2,800	1.33
13	<i>Polytoma papillatum</i>	Green	930	0.74
14	<i>Scenedesmus falcatus</i>	Green	720	0.95
15	<i>Scenedesmus protuberans</i>	Green	1,100	0.79
16	<i>Scenedesmus quadricauda</i>	Green	1,234	0.96
17	<i>Scenedesmus</i> sp.	Green	520	1.03
18	<i>Selenastrum bibraianum</i>	Green	131	0.84
19	<i>Synechocystis aquatilis</i>	Blue-green	50	1.85

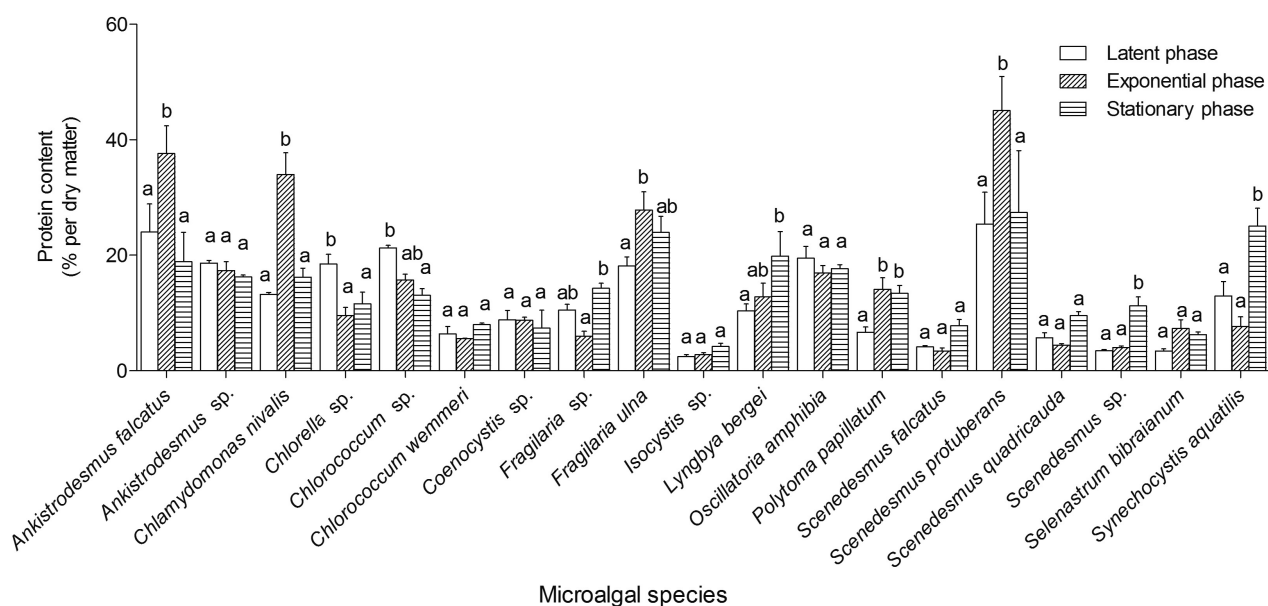


**Fig. 3:** Dispersion of studied microalgal species according to growth rate ( $d^{-1}$ ) and individual biovolume ( $\mu m^3$ ) (▲ Green algae, ■ Cyanobacteria, ● Diatoms). 1: *Ankistrodesmus falcatus*; 2: *Ankistrodesmus* sp.; 3: *Chlamydomonas nivalis*; 4: *Chlorella* sp.; 5: *Chlorococcum* sp.; 6: *Chlorococcum wemmeri*; 7: *Coenocystis* sp.; 8: *Fragilaria* sp.; 9: *Fragilaria ulna*; 10: *Isocystis* sp.; 11: *Lyngbya bergei*; 12: *Oscillatoria amphibian*; 13: *Polytoma papillatum*; 14: *Scenedesmus falcatus*; 15: *Scenedesmus protuberans*; 16: *Scenedesmus quadricauda*; 17: *Scenedesmus* sp.; 18: *Selenastrum bibrainum*; 19: *Synechocystis aquatilis*

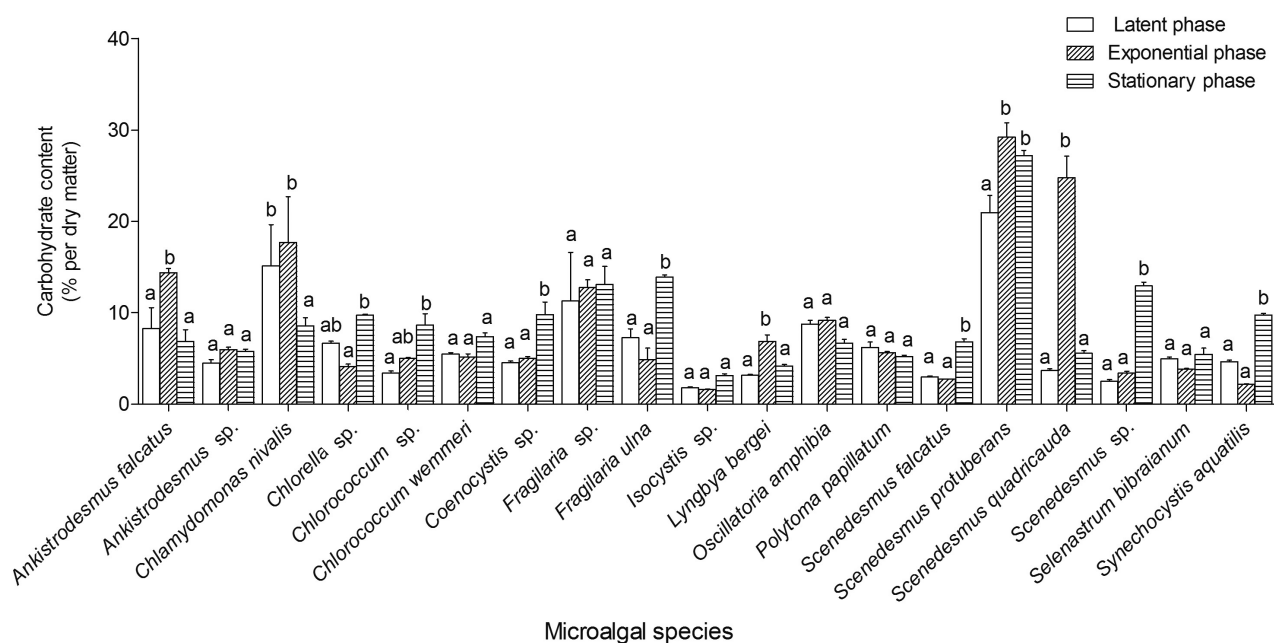
higher content for analyzed biochemical components at each growth phase are summarized in Table 4.

Protein content increased significantly during the exponential growth phase for *Ankistrodesmus falcatus*, *Chlamydomonas nivalis*, *Fragilaria ulna* and *Scenedesmus protuberans*, while for *Fragilaria* sp., *Lyngbya bergei*, *Scenedesmus* sp. and *Synechocystis aquatilis* it was most pronounced in the stationary growth phase. In the

case of *Polytoma papillatum*, protein content was higher in both growth phases. Our results also show that protein content in 8 species (42.1% of all studied species) was stable during the different growth phases. The highest ( $P < 0.05$ ) protein content (45.05% dry weight: DW) was found in *Scenedesmus protuberans*, in the exponential phase, and the lowest ( $P < 0.05$ ) in *Isocystis* sp. (2.42 to 4.21% DW), independently of the growth phase.



**Fig. 4:** Variation in protein content (% of dry matter) depending on growth stage for different microalgal species. The different letters indicate significant differences according to Bonferroni test at  $p < 0.05$ . Vertical bars indicate standard deviations ( $\pm$ ) for the 3 repetitions.



**Fig. 5:** Variation in carbohydrate content (% of dry matter) depending on growth stage for different microalgal species. Different letters indicate significant differences according to Bonferroni test at  $p < 0.05$ . Vertical bars indicate standard deviations ( $\pm$ ) for the 3 repetitions.

**Table 3.** Biochemical composition of the studied microalgal species and certain species reported in the literature.

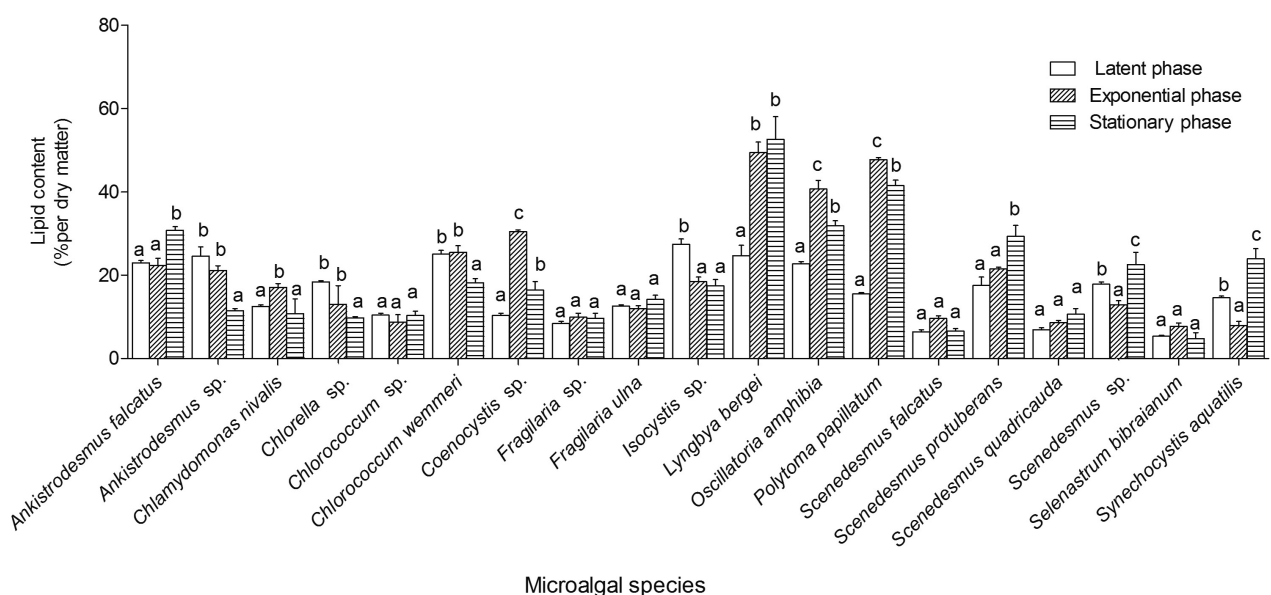
Microalgal species	% of proteins (DW)	% of carbohydrates (DW)	% of lipids (DW)	Reference
<i>Ankistrodesmus falcatus</i>	18.91 – 37.60	6.85 – 14.39	22.29 – 30.79	This study
<i>Ankistrodesmus</i> sp.	16.24 – 18.66	4.48 – 5.97	11.48 – 24.51	
<i>Chlamydomonas nivalis</i>	13.20 – 33.98	8.57 – 17.69	10.77 – 17.06	
<i>Chlorella</i> sp.	9.55 – 18.47	4.13 – 9.76	9.80 – 18.36	
<i>Chlorococcum</i> sp.	13.08 – 21.27	3.42 – 8.67	8.71 – 10.44	
<i>Chlorococcum wemmeri</i>	5.59 – 8	5.13 – 7.38	18.18 – 25.53	
<i>Coenocystis</i> sp.	7.42 – 8.84	4.55 – 9.80	10.37 – 30.44	
<i>Fragilaria</i> sp.	5.98 – 14.26	11.29 – 13.10	8.39 – 9.93	
<i>Fragilaria ulna</i>	18.17 – 27.83	4.88 – 13.90	11.93 – 14.16	
<i>Isocystis</i> sp.	2.42 – 4.21	1.61 – 3.13	17.41 – 27.42	
<i>Lyngbya bergei</i>	10.39 – 19.85	3.16 – 6.87	24.65 – 52.60	
<i>Oscillatoria amphibia</i>	16.94 – 19.48	6.67 – 9.17	22.69 – 40.67	
<i>Polytoma papillatum</i>	6.66 – 14.09	5.20 – 6.21	15.46 – 47.74	
<i>Scenedesmus falcatus</i>	3.37 – 7.83	2.73 – 6.83	6.41 – 9.60	
<i>Scenedesmus protuberans</i>	25.40 – 45.05	20.95 – 29.21	17.53 – 29.30	
<i>Scenedesmus quadricauda</i>	4.38 – 9.55	3.67 – 24.76	6.91 – 10.60	
<i>Scenedesmus</i> sp.	3.45 – 11.21	2.51 – 12.95	12.88 – 22.56	
<i>Selenastrum bibrainum</i>	3.41 – 7.29	3.83 – 5.45	4.79 – 7.73	
<i>Synechocystis aquatilis</i>	7.66 – 25.06	2.14 – 9.74	7.90 – 23.92	
<i>Aphanothice microscopia</i>	41.3 – 49.3	13.4 – 17.6	7.1 – 7.9	Zepka et al., 2007
<i>Botryococcus braunii</i>	–	–	20.75	Yoo et al., 2010
<i>Chlorella protothecoides</i>	52.64	10.62	14.57	Xu et al., 2006
<i>Chlorella vulgaris</i>	51–58	12–17	14–22	Lum et al., 2013
<i>Cryptomonas</i> sp.	44.2 – 50	3.9 – 4.5	19.6 – 21.4	Renaud et al., 2001
<i>Rhodomonas</i> sp.	53.7 – 58.8	6 – 7.8	8 – 12.7	
<i>Chlamydomonas reinhardtii</i>	48	17	21	Um and Kim 2009
<i>Chlorella pyrenoidosa</i>	57	26	2	
<i>Scenedesmus dimorphus</i>	8–18	21–52	16–40	
<i>Scenedesmus obliquus</i>	50–56	10–17	12–14	
<i>Scenedesmus quadricauda</i>	47	–	1.9	

**Table 4.** Summary of the studied microalgal species with higher content for analyzed biochemical components in each growth phase.

	Latent phase	Exponential phase	Stationary phase	Indifferent
Proteins	<i>Chlorella</i> sp. <i>Chlorococcum</i> sp.	<i>Ankistrodesmus falcatus</i> <i>Chlamydomonas nivalis</i> <i>Fragilaria ulna</i> <i>Polytoma papillatum</i> <i>Scenedesmus protuberans</i>	<i>Fragilaria</i> sp. <i>Lyngbya bergei</i> <i>Polytoma papillatum</i> <i>Scenedesmus</i> sp. <i>Synechocystis aquatilis</i>	<i>Ankistrodesmus</i> sp. <i>Chlorococcum wemmeri</i> <i>Coenocystis</i> sp. <i>Isocystis</i> sp. <i>Oscillatoria amphibia</i> <i>Scenedesmus falcatus</i> <i>Scenedesmus quadricauda</i> <i>Selenastrum bibraianum</i>
Carbohydrates	<i>Chlamydomonas nivalis</i>	<i>Ankistrodesmus falcatus</i> <i>Chlamydomonas nivalis</i> <i>Lyngbya bergei</i> <i>Scenedesmus protuberans</i> <i>Scenedesmus quadricauda</i>	<i>Ankistrodesmus falcatus</i> <i>Chlorella</i> sp. <i>Chlorococcum</i> sp. <i>Coenocystis</i> sp. <i>Fragilaria ulna</i> <i>Scenedesmus protuberans</i> <i>Scenedesmus</i> sp. <i>Synechocystis aquatilis</i>	<i>Ankistrodesmus</i> sp. <i>Chlorococcum wemmeri</i> <i>Fragilaria</i> sp. <i>Isocystis</i> sp. <i>Oscillatoria amphibia</i> <i>Polytoma papillatum</i> <i>Selenastrum bibraianum</i>
Lipids	<i>Ankistrodesmus</i> sp. <i>Chlorella</i> sp. <i>Chlorococcum wemmeri</i> <i>Isocystis</i> sp.	<i>Ankistrodesmus</i> sp. <i>Chlamydomonas nivalis</i> <i>Chlorella</i> sp. <i>Chlorococcum wemmeri</i> <i>Coenocystis</i> sp. <i>Lyngbya bergei</i> <i>Oscillatoria amphibia</i> <i>Polytoma papillatum</i>	<i>Ankistrodesmus falcatus</i> <i>Lyngbya bergei</i> <i>Scenedesmus protuberans</i> <i>Scenedesmus</i> sp. <i>Synechocystis aquatilis</i>	<i>Ankistrodesmus falcatus</i> <i>Chlorococcum</i> sp. <i>Fragilaria</i> sp. <i>Fragilaria ulna</i> <i>Scenedesmus quadricauda</i> <i>Selenastrum bibraianum</i>

In *Ankistrodesmus falcatus*, *Chlamydomonas nivalis*, *Scenedesmus protuberans* and *Scenedesmus quadricauda*, carbohydrate content reached its highest level during the exponential phase (14.34%, 21.81%, 29.21% and 24.76% DW, respectively), while in *Ankistrodesmus falcatus*, *Chlorella* sp., *Chlorococcum* sp., *Coenocystis* sp., *Fragilaria ulna*, *Scenedesmus protuberans*, *Scenedes-*

*mus* sp. and *Synechocystis aquatilis*, this biochemical compound increased significantly in the stationary phase. In *Ankistrodesmus* sp., *Chlorococcum wemmeri*, *Fragilaria* sp., *Isocystis* sp., *Oscillatoria amphibia*, *Polytoma papillatum* and *Selenastrum bibraianum*, carbohydrate content showed no statistical differences between growth phases.



**Fig. 6:** Variation in lipid content (% of dry matter) depending on growth stage for different microalgal species. Different letters indicate significant differences according to Bonferroni test at  $p < 0.05$ . Vertical bars indicate standard deviations ( $\pm$ ) for the 3 repetitions.



Lipid content increased significantly during the exponential growth phase for *Chlamydomonas nivalis*, *Coenocystis* sp., *Lyngbya bergei*, *Oscillatoria amphibian* and *Polytoma papillatum* (17.06%, 30.44%, 49.40%, 40.67% and 47.74% DW, respectively). The highest lipid content was found also in *Ankistrodesmus falcatus*, *Lyngbya bergei*, *Scenedesmus protuberans*, *Scenedesmus* sp. and *Synechocystis aquatilis* in the stationary phase. The highest (52.60% DW) and lowest (4.79% DW) lipid levels were found in *Lyngbya bergei* and *Selenastrum bibraianum*, respectively, during the stationary phase.

## Discussion

The range in growth rates of the nineteen microalgal species, overlapping from 0.27 d<sup>-1</sup> to 3.64 d<sup>-1</sup>, reflects the ecophysiological differences among species. The fundamental cultivation factors determining microalgae growth rate are nutrient quality and quantity (Koller *et al.*, 2012). Thus, in this study we reported growth stabilization after three weeks of culture, which was likely due to the depletion in the culture medium nutrient concentrations, especially nitrogen and phosphorus (Boumnick *et al.*, 2001). Furthermore, a wide range of decisive cultivation factors (e.g., light supply, light intensity, salinity, pH, turbulence and temperature) determines microalgal growth and product formation rates (Aleya *et al.*, 2011;

Stengel *et al.*, 2011; Koller *et al.*, 2012), factors which not only affect photosynthesis and algae growth rate, but also influence cellular metabolism and composition (Aleya *et al.*, 1992; Juneja *et al.*, 2013; Borderie *et al.*, 2014 a,b). In the present study, however, it was not possible to discern the contributing effects of such factors.

Exploitation of microalgae as a protein source has led to increased interest in their use (e.g., *Spirulina*, *Chlorella* and *Scenedesmus*) in health food production (Becker, 2007). The values obtained reveal that, due to interspecies variations (Morris, 1981), protein levels can vary from one species to another. Protein content differed also for the same species according to growth phase, in agreement with (Belkoura *et al.* 1997). In the present study, protein levels varied from 45.05% DW during the exponential phase to 25.40% DW in the latent phase for the green algae *Scenedesmus protuberans*, and from 4.21% DW during the stationary phase to 2.42% DW in the lag phase for the blue-green algae *Isocystis* sp. The species in the present study showed a lower protein content compared to those reported in the literature, with values exceeding 41.3%, except for *Scenedesmus dimorphus* for which the reported protein content ranges from 8 to 18% DW (Um & Kim, 2009). This may be due to temperature (Renaud *et al.*, 2001; Zepka *et al.*, 2007), light intensity and photoperiod (George *et al.*, 2014), light quality (Morris, 1981), nature and concentration of

the nitrogen source (Pancha *et al.*, 2014), osmotic pressure of the culture medium (Ben-Amotz *et al.*, 1985) or organic matter intake (Endo *et al.*, 1974).

Carbohydrate concentrations also vary greatly between and within taxa, depending on growth phases (Phlips *et al.*, 1989). In the present study they varied from 29.21% DW during the exponential phase to 20.95% DW in the lag phase for *Scenedesmus protuberans*, and from 3.13% DW during the stationary phase to 1.61% DW during the exponential phase in *Isocystis* sp. Um & Kim (2009) found that the carbohydrate content in microalgae can reach up to 52% of total cell mass of *Scenedesmus dimorphus*; in our study, the dry weight of carbohydrates was very low compared to that of protein and lipid, similar to those obtained in earlier studies of algae in culture. Factors such as protein production, environmental and nutritional factors can affect the carbohydrate content in different microalgal species. Researchers have identified the individual effects of these factors (Morris, 1981; Zepka *et al.*, 2007; George *et al.*, 2014; Pancha *et al.*, 2014), though no consolidated review is available as to their effects on microalgae. Therefore, it is very difficult, if not impossible, to draw definitive conclusions about any factor influencing the carbohydrate biosynthesis processes in algae. Concerning economic interests, carbohydrates such as starch can be easily converted to ethanol by hydrolysis and fermentation (Juneja *et al.*, 2013). Algal starch is known to be readily fermentable by yeast (Nguyen *et al.*, 2009) and, therefore, is under intense study for use in ethanol production (John *et al.*, 2011).

An essential component of membrane systems, lipid content also varies according to species. Thus, the changes observed in the lipid content in proteins in the nineteen studied species are due both to interspecies variations (Borowitzka, 1988) and to the growth stage (Barclay *et al.*, 1985). Total lipid contents varied from 24.65% DW registered during the lag phase to 52.60% in the stationary phase for the cyanobacterium *Lyngbya bergei*, and from 4.79% DW measured in the stationary phase to 7.73% DW during the exponential phase for the green algae *Selenastrum bibraianum*. Thus, total lipid content in the species studied here was higher than those reported in the literature. In addition, this variability is possibly influenced by cultivation factors, such as temperature (Zepka *et al.*, 2007), light intensity and photoperiod (George *et al.*, 2014), CO<sub>2</sub> concentration of the culture medium (Yoo *et al.*, 2010), nature and concentration of the nitrogen source (Pancha *et al.*, 2014), iron concentration (Liu *et al.*, 2008), silica concentration in the case of diatoms (Aleya *et al.*, 1992), osmotic pressure in the medium (Al Hassan *et al.*, 1990) and degree of culture aeration (Chen & Johns, 1991). Juneja *et al.* (2013) in a recent and highly informative review presented abundant data on the impacts of these multiple environmental and nutritional factors, which indicates the current interest

for lipid production in microalgae, specifically in relation to biofuel production.

## Conclusion

The present study shows that the taxa *Ankistrodesmus falcatus*, *Chlamydomonas nivalis*, *Fragilaria ulna*, *Scenedesmus protuberans* and *Synechocystis aquatilis* are a promising feedstock for potential protein sources for health food production, while the species *Scenedesmus protuberans* and *Scenedesmus quadricauda* both have a tremendous potential in carbohydrate production. Lipid content in *Lyngbya bergei*, *Oscillatoria amphibia* and *polytoma papillatum* can be as high as 40% (dry-weight), therefore showing their high capacity for lipid production. These microalgal species may thus have a role to play in the development of alternative energy sources. However, great care must be taken so as to identify the most promising species; in this process, the multitude of environmental and nutritional factors impacting algal growth and broader metabolic regulation must be considered. A better understanding of these factors is critical for the successful promotion of algae cultures in commercial systems in order to maximize chemical constituent production.

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## References

Aleya, L., 1989. Seasonal couplings between adenyl nucleotides and photosynthetic activity of size-fractionated phytoplankton in a eutrophic lake. *European Journal of Protistology*, 24, 381-391.

Aleya, L., 1992. The seasonal succession of phytoplankton in an eutrophic lake through the coupling of biochemical composition of particulates, metabolic parameters and environmental conditions. *Fundamental and Applied Limnology*, 124, 69-88.

Aleya, L., Dauta, A., Reynolds, C.S., 2011. Endogenous regulation of the growth-rate responses of a spring-dwelling strain of the freshwater alga, *Chlorella minutissima*, to light and temperature. *European Journal of Protistology*, 47, 239-244.

Al Hassan, R.H., Ali A.M., Kawash, H.H., Radwan, S.S., 1990. Effect of salinity on the lipid and fatty acid composition of the halophyte *Navicula* sp.: potential in mariculture. *Journal of Applied Phycology*, 2, 215-222.

Andersen, R., 2005. Algal culturing techniques. Elsevier Academic Press, 2005, Phycological Society of America, Hing Yip Co, China, 589 pp.

Barclay, B., Nagle, N., Terry, K., Roessler, P., 1985. Collecting and screening microalgae from shallow, inland saline habitats. *SERI Aquatic Species Review*, SERI/CP-23-2700, 52-68.

Becker, E.W., 2007. Micro-algae as a source of protein. *Biotechnology Advances*, 25, 207-210.

Becker, E.W., 1988. Micro-algae for human and animal consumption. In *Micro-algal biotechnology*. Borowitzka M.A. & Borowitzka L.J. (Eds.), Cambridge University Press, London, pp. 222-256.

Beyerinck, M.W., 1890. *Culturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen Algen*. *Botanische Zeitung* 47, 725-739, 741-754, 757-768, 781-785.

Belkoura, M., Benider, A., Dauta, A., 1997. Influence de la température, de l'intensité lumineuse et du stade de croissance sur la composition biochimique de *Chlorella sorokiniana* Shihira & Krauss. *Annales de Limnologie*, 33, 3-11.

Ben-Amotz, A., Tornabene, T.G., Thomas W.H., 1985. Chemical profile of selected species of microalgae with emphasis on lipids. *Journal of Phycology*, 21, 72-81.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.

Borderie, F., Tête, N., Cailhol, D., Alaoui-Sehmer, L., Boust, F. et al., 2014a. Factors driving epilithic algae colonization in show caves and new insights into how to combat biofilm development through UV-C treatments. *Science of the Total Environment*, 484, 43-52.

Borderie, F., Alaoui-Sehmer, L., Boust, F., Alaoui-Sossé, B., Aleya, L., 2014b. Cellular and molecular damage caused by high UV-C irradiation of the cave-harvested green alga *Chlorella minutissima*: implications for cave management. *International Biodeterioration and Biodegradation*, 93, 118-130.

Borowitzka, M.A., 1988. Fats, oils and hydrocarbons. In *Microalgal biotechnology*. Borowitzka M.A. & Borowitzka L.J. (Eds.), Cambridge University Press, London. pp 257-287.

Boumnic, L., Derraz, M., Naji, B., Dauta, A., 2001. Influence des facteurs héliothermiques et nutritionnels sur la croissance et les capacités de stockage intracellulaire (azote et phosphore) chez *Microcystis aeruginosa* Kützinger isolée de la retenue eutrophe El Kansera (Maroc). *Annales de Limnologie*, 37, 191-198.

Brunel-Delclaux, L., Guerri, M.M., 1980. *Cinétiques d'assimilation et de croissance de Scenedesmus crassus: étude expérimentale et modélisation*. Thèse 3<sup>ème</sup> cycle, Toulouse.

Chen, C.Y., Yeh K.L., Aisyah, R., Lee D.J., Chang J.S., 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Biore-source Technology*, 102, 71-81.

Chen F., Johns M., 1991. Effect of C/N ratio and aeration on the fatty acid composition of heterotrophic *Chlorella sorokiniana*. *Journal of Applied Phycology*, 3, 203-209.

- Dubois, M., Gilles, K.A., Hamilton, J.K., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28, 350-356.
- El-Fouly, M.M., Mohn, F.H., Soeder, C.J., 1985. Joint Egyptian- German project. Intensive protein production through microalgae. *Kernforschungsanlage Jilich GmbH, Publication*, 34/85.1.
- Endo, H., Nakajima, K., Chino, R., Shiota, M., 1974. Growth characteristics and cellular components of *Chlorella regularis*, heterotrophic fast growing strain. *Agricultural and Biological Chemistry*, 38, 9-18.
- George, B., Pancha, I., Desai, C., Chokshi, K., Paliwal, C. *et al.*, 2014. Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus* – A potential strain for bio-fuel production. *Bioresource Technology*, 171, 367-374.
- Gross, R., Schoneberger, H., Gross, U., Lorenzen, H., 1982. The nutritional quality of *Scenedesmus acutus* produced in a semiindustrial plant in Peru. *Berichte der Deutschen Botanischen Gesellschaft*, 95, 323-327.
- Hillebrand, H., Du'r'selen, C.D., Kirschtel, D., Pollinger, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, 35, 403-424.
- Hsieh, C.H., Wu, W.T., 2009. A novel photobioreactor with transparent rectangular chambers for cultivation of microalgae. *Biochemical Engineering Journal*, 46, 300-305.
- John, R.P., Anisha, G., Nampoothiri, K.M., Pandey, A., 2011. Micro and macroalgal biomass: A renewable source for bioethanol. *Bioresource Technology*, 102, 186-193.
- Juneja, A., Ceballos, R.M., Murthy, G.S., 2013. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review. *Energies*, 6, 4607-4638.
- Khattabi, H., Aleya, L., Mania, J., 2006. Spatio-temporal distribution and characterisation of phytoplankton populations coupled with abiotic and biotic changes in landfill leachate treatment basins (Etuffont, Belfort, France). *Water, Air and Soil Pollution*, 174, 107-125.
- Koller, M., Salerno, A., Tuffner, P., Koinigg, M., Böchzelt, H. *et al.*, 2012. Characteristics and potential of micro algal cultivation strategies: a review. *Journal of Cleaner Production*, 37, 377-388.
- Liu, Z.Y., Wang, G.C., Zhou, B.C., 2008. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresource Technology*, 99, 4717-4722.
- Lum, K.K., Kim, J., Lei, X.G., 2013. Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. *Journal of Animal Science and Biotechnology*, 4, pp 53.
- Miquel P., 1890/92. De la culture artificielle des Diatomées. *Le Diatomiste* 1, 73-5, 93-9, 121-8, 149-56, 165-72.
- Morris, I., 1981. Photosynthetic products, physiological state, and phytoplankton growth. In Physiological bases of Phytoplankton Ecology. Piatt T. (Ed.). *Canadian Bulletin of Fisheries and Aquatic Sciences*, 210, 83-102.
- Nguyen, M.T., Choi, S.P., Lee, J., Lee, J.H., Sim, S.J. 2009. Hydrothermal acid pretreatment of *Chlamydomonas reinhardtii* biomass for ethanol production. *Journal of Microbiology and Biotechnology*, 19, 161-166.
- Pancha, I., Chokshi, K., George, B., Ghosh, T., Paliwal, C. *et al.*, 2014. Nitrogen stress triggered biochemical and morphological changes in the microalgae *Scenedesmus* sp. CCNM 1077. *Bioresource Technology*, 156, 146-154.
- Phlips, E.J., Zeman, C., Hansen, P., 1989. Growth, photosynthesis, nitrogen fixation and carbohydrate production by a unicellular cyanobacterium, *Synechococcus* sp. (Cyanophyta). *Journal of Applied Phycology*, 1, 137-145.
- Renaud, S.M., Luong-Van, T., Lambrinidis, G., Parry D.L., 2001. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture*, 211, 195-214.
- Renaud, S.M., Luong-Van, T., Parry, D.L., 1999. The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. *Aquaculture*, 170, 147-159.
- Reynolds, C.S., 1997. Vegetation processes in the pelagic: a model for ecosystem theory (Excellence in Ecology 9). Ecology Institute, Oldendorf, 371 pp.
- Simmer, J., 1969. Outdoor mass cultivation of *Scenedesmus quadricauda* (Turp.) Breb. in South Bohemia. In Studies in Phycology. Fort B., (Ed.), E. Schweitzerbartsche Verlagsbuchhandlung, Stuttgart, 293-304.
- Stengel, D.B., Connan, S., Popper, Z.A., 2011. Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. *Biotechnology Advances*, 29, 483-501.
- Um, B.H., Kim, Y.S., 2009. A chance for Korea to advance algal-biodiesel technology. *Journal of Industrial and Engineering Chemistry*, 15, 1-7.
- Xu, H., Miao, X., Wu, Q., 2006. High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *Journal of Biotechnology*, 126, 499-507.
- Yoo, C., Jun, S.Y., Lee, J.Y., Ahn, C.Y., Oh H.M., 2010. Selection of microalgae for lipid production under high levels carbon dioxide. *Bioresource. Technology*, 101, S71-S74.
- Zepka, Q.L., Jacob-Lopes, E., Goldbeck, R., Queiroz, M.I., 2007. Production and biochemical profile of the microalgae *Aphanothece microscopica* Nageli submitted to different drying conditions. *Chemical Engineering and Processing: Process Intensification*, 47, 1305-1310.